CHARACTERIZATION AND COMPARISON OF EXOSOMES FOUND IN STEM CELL CULTURES TO THOSE FOUND IN PLANT BOTANICALS ESPECIALLY COCOS NUCIFERA

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Characterization and Comparison of Exosomes Found in Stem Cell Cultures to Those Found in Plant Botanicals Especially Cocos Nucifera

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Abstract

While extensive information is published regarding human exosomes derived from adult stem cell cultures, limited publications are available on exosomes extracted from plant botanicals. This review characterized that similar to exosomes derived from adult stem cultures, exosomes extracted from plant botanicals such as *cocos nucifera* (coconut) water have demonstrated similar biological activities yet through exerting cross-species plant-to-human exosome-mediated intercellular communication and development. Plant exosomes, characterized by their differential advantages compared to mammalian-derived exosomes, are considered as more ideal exosomal sources regarding its scalability and cultivation convenience. Due to smaller exosomal size, coconut water in particular is characterized with more efficient absorption/uptake by human cells and delivery of exosomal contents (e.g., mircroRNAs) upon exerting biological activity against human cells, which potentially enlighten future applications of coconut water exosome-based therapeutics in humans.

Keywords

Exosome, Plant exosome, *Cocos nucifera*, Coconut water, microRNA, Stem cell cultures, Exosome isolation, Cross-species, Regenerative medicine.

Introduction

Exosomes, nanosized with an average of approximately 100 nm in diameter, are widely recognized as a subset of extracellular vesicles (EVs) of endocytic origin, secreted by all cell types and have been found in birth tissues such as amniotic fluid (AF) [1-3]. Exosomes are loaded with many diverse functional cargo constituents including proteins (cytosolic and cell-surface proteins/receptors, transcription factors, enzymes), nucleic acids (DNAs, RNAs, particularly microRNAs (miRNAs)) [4], lipids (cholesterols, saturated fatty acids), amino acids, and metabolites (Figure 1) [3, 5]. The ExoCarta (http://www.exocarta.org) database is an excellent resource for exosome-based studies, documented at least 41,860 exosomal protein, 1,116 exosomal lipid molecules, and 4,949 exosomal RNAs (including 2,838 exosomal miRNAs) [5, 6]. Recently, exosomal miRNAs have attracted great interests from researchers because both plant and

mammalian exosomes are found to alter gene expression due to transfer of miRNAs that can regulate endogenous gene expression in humans, at least in the *in vitro* systems [4, 7].

Exosomes can function as therapeutic agents by themselves or as therapeutic vehicles for delivery of functional cargos to human recipient cells and heal necrotic/apoptotic cells of the connective tissue [8]. As dynamic mediators of intercellular communication both locally and distantly, exosomes can be released into extracellular space and internalized by recipient cells, delivering exosomal cargo that can be absorbed by neighboring or distant recipient cells, eliciting downstream signals in recipient cells to regulate biological processes [1, 3]. Once recipient cells uptake exosomal cargo through their fusion with the plasma membrane during endocytotic or phagocytotic internalizations, exosomes can transfer/deliver functional cargos to effect/alter recipient cells phenotypically, physiologically, and pathologically via dynamic exosome-mediated intercellular communication [1, 3, 9, 10]. For instance, exosomes may "detoxify" cells by removing unnecessary constituents from cells to maintain cellular homeostasis [1]. Although the exosomal proteome is determined by the proteome of the originating cell, the exosomal functional cargos can be altered/impacted by the type of exosomes, the microenvironment, and the nature of recipient cells (e.g., the recipient cell's level of expression on the surface antigen repertoires) [1, 3]. The rich exosomal composition, along with the capacity to interact between exosomes and surface antigens of recipient cells, appear to be primarily responsible to induce the extent of internalization and subsequent functional roles in proliferation [3]. Interestingly, such proliferative and regenerative effects were also exerted by exosomes derived from adult stem cell cultures found in birth tissues such as the amniotic fluid (AF), as demonstrated by the isolated exosomes with high cell-type specificity, characterizing various putative functions including cardioprotective activity and cutaneous wound healing [3, 11]. An efficient exchange of such cellular components via exosomes may potentially be applied in designing exosome-based therapeutics in humans [1].

Recently, the functional and targeted accumulation of specific cellular components are found in exosomes derived from adult stem cell cultures found in AF [12, 13]. Moreover, the cytokines and growth factors in the non-cellular fraction of AF are critical in regenerative treatments in humans [14]. Importantly, the number of exosomes collected are driven by the isolation techniques used, the kinds of exosomes, and the biological activities of such exosomes *in vitro* or *in vivo* [12]. Exosomes can be isolated from collecting conditioned media (supernatant) of cell cultures followed by serial centrifugations (300*g* for 10 min, 2,000*g* for 10 min and 10,000*g* for 30 min at 4 °C) [15]. The supernatant from the 10,000*g* centrifugation was ultracentrifuged at 100,000*g* for 70 min at 4 °C to collect the exosome-enriched fraction (pellet) [12]. The preferred isolation technique of exosomes derived from adult stem cell cultures found in AF is size-exclusion chromatography (qEV), which has generated higher yield of exosomes, lower protein contamination, lower sheer stress on EVs, and higher efficiency with approximately 15 minutes per preparation [12]. In contrast, the ultracentrifugation (UC) technique has inconsistent reproducibility (due to rotor size, UC time, speed, and temperature), less purity of EV pellet (due to aggregation of other particles), and less efficiency overall (processing time may take more than 12 hours) [12]. Other isolation techniques such as ExoQuick, TEIR (Total Exosome Isolation Reagent), and Exo-PREP techniques have drawbacks regarding the undesirable cost per preparation and the retention of polymers in reagent [12]. Regarding exosomal yield, the UC

isolation technique has yielded higher number of exosomes of approximately 3×10^7 exosomes per milliliter [12]. Therefore, standardizing the isolation techniques of exosomes are critical to minimize lot-to-lot variations since the exosomal yield could be impacted by the exosomal size, expression of surface biomarkers, purity and acceptable contamination levels for identification and quality control of the isolated exosomes.

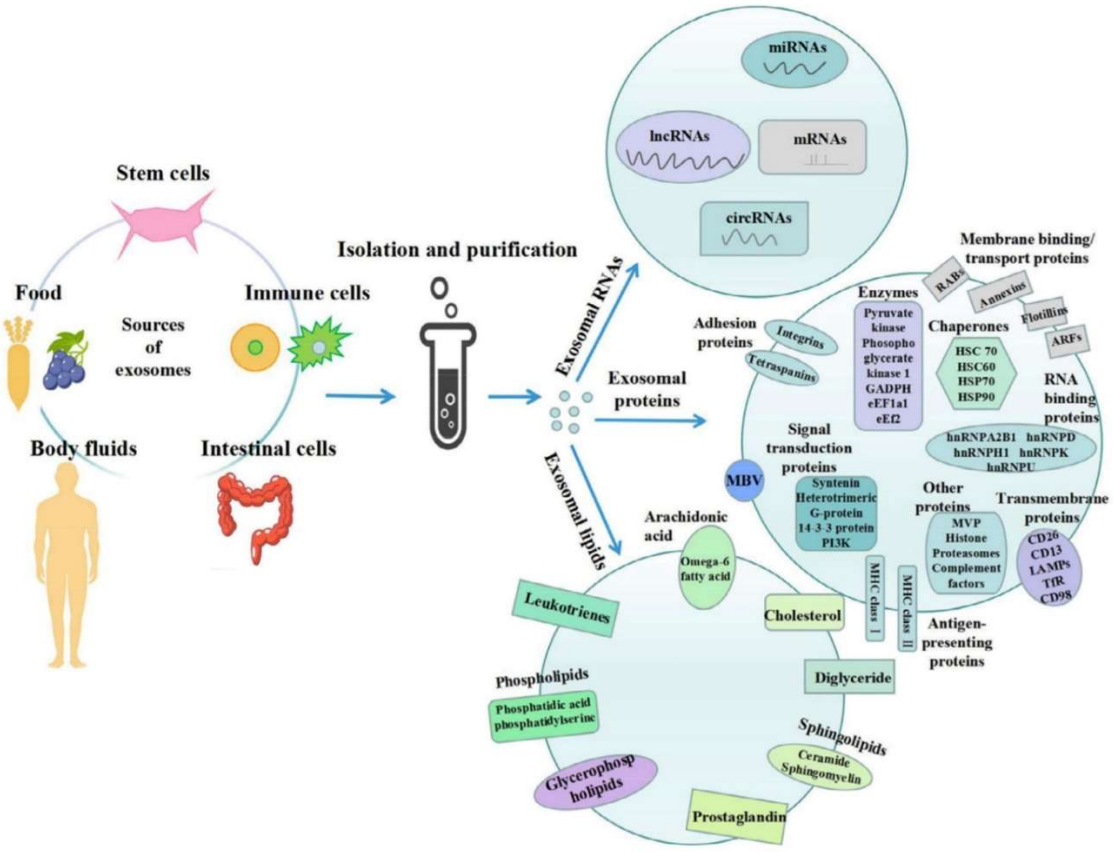
While exosomes that derived from adult stem cell cultures of different origins might differ in exosomal contents and their corresponding biological functions on target recipient cells, exosomes by themselves can exert biological functions similar to the adult stem cell cultures that they have derived from [10]. The biological activities of such exosomes are characterized by the induction of damaged tissue repair and the cellular uptake of exosomes by the recipient cells, promoting cellular activities such as migration and proliferation via adenosine receptor activation of phosphatidylinositol 3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathways, both are important cellular signaling cascades with large functional overlaps in cell growth, proliferation and survival/apoptosis, and are both activated by receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs) [13, 16]. Nonetheless, exosomes derived from adult stem cell cultures are challenged by the scalability in large-scale applications due to limited sourcing and supplies [17]. As such, alternative exosomal sources are worthy of characterization and comparison, with exosomes extracted from plant botanicals in particular [17]. The purpose of this review is to characterize and compare exosomes extracted from plant botanicals, especially coconut water, to exosomes derived from adult stem cell cultures found in AF. This review is to elucidate (1) whether plant exosomes could potentially substitute mammalian exosomes by characterizing and comparing between both exosomal sources, and (2) whether plant exosomes can exert biological activities in humans (e.g., human cell proliferation, tissue regeneration, differentiation) through cross-species plant-to-human exosome-mediated intercellular communication.

Although plants are recognized as stimulants for stem cell proliferation by their production of active cosmetic ingredients, the key element that contribute to this activity was not well understood [18]. Although extracts from plants are already widely used as effective active ingredients in cosmeceuticals such as the skin-care formulations through topical applications, the cosmeceutical industry has not been focusing on the isolation and characterization of the key contributing element, i.e., exosomes [19]. Indeed, plant cell culture-derived active cosmetic ingredients with more enriched exosomes have revealed stronger activities than the plant extracts obtained by the traditional methods that are applied by majority of the cosmeceuticals [19]. While extensive publications are available on exosomes derived from adult stem cell cultures found in birth tissues such as AF, limited papers are available on plant exosomes. Compared to exosome derived from adult stem cell cultures, exosomes extracted from plant botanicals are characterized by similar biological activities to alter target human gene expressions through absorption in the human gastrointestinal (GI) tract, mediating plant-to-mammal intercellular communication while retaining exosomal stability [22]. Moreover, plant exosomes are characterized of their additional benefits including their (1) tissue-specific targeting activities with significantly lower allergenic effects and immunogenicity; (2) natural and biodegradable resources, leading to better

environmental safety; and (3) renewable and sustainable resources, leading to greater prospects for industrial large-scale production [4, 17, 20, 21].

Among the exosomes extracted from plant botanicals, cocos nucifera (coconut) water is reviewed in detail pertaining to exosomal size, exosomal miRNAs and biological processes. Compared to other plant exosomes, coconut water is selected due to its differential advantage of (1) smaller exosomal size compared to many other plant exosomes as well as mammalian exosomes, leading to more efficient absorption and recipient cellular uptake; (2) exosome isolation convenience from coconut water, with no grinding and extraction needed, compared to majority of other plant botanicals where grinding and extraction is necessary; and (3) well-recognized functional health properties and medicinal benefits [22, 23]. This review is to characterize and compare exosomes isolated from coconut water against exosomes extracted from plant botanicals and exosomes derived from adult stem cell cultures found in AF by comparing their exosomal size. Furthermore, the purpose of this review is to demonstrate that exosomes extracted from plant botanicals, particular coconut water, are characterized of similar biological activities to exosomes derived from adult stem cell cultures found in AF through intercellular communications and alteration of gene expressions in humans, modulating both physiological and pathological activities via vesicular transport and delivery of functional units to recipient human cells [8].

Figure 1: Characterization of exosomal sources and compositions [5]



Methodology

Database searches of national center for biotechnology information (NCBI) including PubMed category and Google Scholar were conducted in the last five years to retrieve articles related to exosomes isolated from adult stem cell cultures and exosomes extracted from plant botanicals including coconut water.

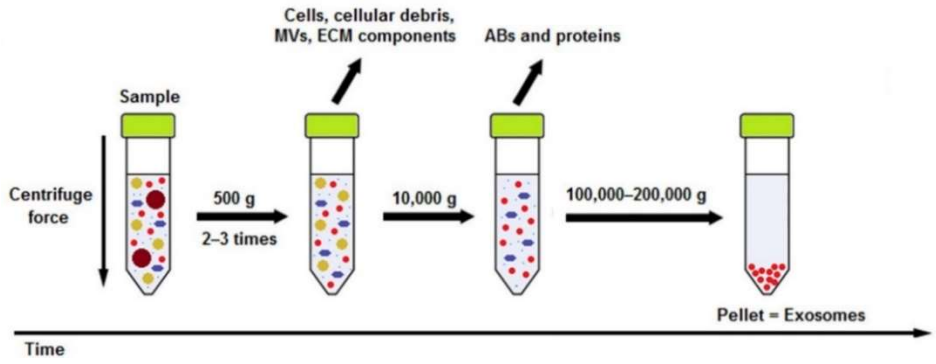
The objective of this research review is to characterize and compare exosomes isolated from *in vitro* adult stem cell cultures to exosomes extracted from plant botanicals especially coconut water that have similar exosomal effects to humans. Such characterization and comparison are centered around the general characteristics and biological activities among the type of exosomes, and how the exosomes are isolated and collected from each of the exosomal sources in discussion.

Results and Discussion

Part 1: Isolation and Collection of Exosomes

To isolate exosomes from *in vitro* human stem cells found in AF, differential ultracentrifugation can be used, as demonstrated by a schematic representation (Figure 2) [9]. Its sample is handled by 2-3 steps of low-speed (500*g*) centrifugation to pellet out cells, microvesicles (MVs), extracellular matrix (ECM) components, and cellular debris [9]. The supernatant is then centrifuged at 10,000*g* to remove apoptotic bodies (ABs) and contaminating proteins [9]. Next, exosomes are collected by a long (1-2 hr) ultracentrifugation at 100,000-200,000*g* to separate exosomes followed by washing the pellet (exosomes) in phosphate buffered saline (PBS) to further purify the exosomes [9]. Upon isolation, the number of exosomes collected are approximately 3×10^7 exosomes/ml [12]. The biological effects of exosomes (e.g., proliferation, differentiation, immune responses, metabolism) are driven by the number of exosomes administered, the type of exosomes, the exosomal isolation techniques, the microenvironment, and the extent of intercellular communications [3].

Figure 2: Schematic representation of exosome isolation method using differential ultracentrifugation [9]



Unlike isolating exosomes from other plant botanicals which grinding needs to be conducted before proceeding to exosomal isolation, the initial grinding step is not needed to isolate exosomes from the naturally available coconut water, which makes coconut water more convenient and efficient to be selected as exosomal source over other plant botanicals. Similar to isolating exosomes from adult stem cell cultures, exosomes are isolated from coconut water (with coconuts purchased in Sichuan, China) using differential centrifugation and ultracentrifugation (UC) [21]. Differential centrifugation is used at 1,200*g* for 20 min, 3,000*g* for 20 min, and 10,000*g* for 60 min at 4 °C to discard large particles and cellular debris. The supernatant is filtered using 1 μm membrane filter followed by UC at 150,000*g* for 90 min at 4 °C. The exosomes are then resuspended in 250 μl phosphate buffer saline (PBS) and are filtered through 0.22 μm membranes [20]. To collect exosomes of better purity, sucrose gradient UC can then be used at 150,000*g* for 120 min [22]. Compared to exosomes derived from adult stem cell cultures, the same isolation and collection methods are widely used although the various isolation techniques for mammalian exosomes are more thoroughly studied and compared pertaining to the advantages and disadvantages of various isolation techniques such as efficiency and exosomal yield (see Introduction).

Part 2: Exosomes Derived from Adult Stem Cell Cultures

Exosomes derived from adult stem cell cultures found in birth tissues such as AF are widely recognized in regenerative medicine due to their potent differentiation, immunomodulatory capacity, and the ability to be cultured and manipulated *in vitro*, making cell-free therapy possible [13, 24].

Mesenchymal stem cells (MSCs), likely originated from the mesoderm, are multipotent nonhematopoietic adult cells and are characterized to proliferate *in vitro* and differentiate into various cell types, making MSCs suitable agents to heal damaged cells of the connective tissue [9, 13-16, 25]. Interestingly, the targeting effects of exosomes can be adjusted by preconditioning MSC culture by adding cytokines [26]. Exosomes derived from adult stem cell cultures in AF are recently reported that can be manipulated and applied to establish cell-free therapeutic approach to target cutaneous wound healing [9]. Importantly, such exosomes are characterized of anti-inflammation characteristics via decreasing messenger RNA (mRNA) levels of pro-inflammatory cytokines (e.g., IL1β, TNFα) while elevating the levels of IL10, an anti-inflammatory cytokine [26]. Specifically, exosomes derived from adult stem cell cultures are characterized to trigger angiogenesis-related biomolecules expression, and can repair wounds by upregulating collage I, proliferating cell nuclear antigen (PCNA), and cell keratin 19 (CK19) expression that exhibit rapid re-epithelialization *in vivo* [24].

Exosomes derived from adult stem cell cultures are already being tested in clinical trials targeting skin-related indications, demonstrating high safety profile with minimal side effects (https://www.clinicaltrials.gov/) [1]. The exosomes derived from adult stem cultures found in AF are found to be efficacious by themselves, and subjects can tolerate well with repeated injections [1]. Moreover, the type of recipient human cells, along with the expression of cell surface markers,

are also critical during the exosome-mediated intercellular communication, as demonstrated by the CD47 protein found on the recipient cell surface, which can mediate growth factor effects on exosomes by releasing a "don't eat me" signal, protecting exosomes from phagocytosis while limiting their clearance from circulation [27]. Overall, these biological effects of exosomes derived from adult stem cell cultures found in birth tissues such as AF are recognized to be beneficial due to (1) the exosome-mediated intercellular communicate to exert potent cellular proliferation, differentiation, and immunomodulatory capacity, as demonstrated by *in vitro* and *in vivo* systems, and (2) administration of exosomes with repeated injections are found to be both safe and efficacious in clinical trials [1].

Nonetheless, exosomes derived from adult stem cell cultures are challenged by the scalability in large-scale applications due to limited sourcing and supplies [17]. As such, alternative exosomal sources extracted from plant botanicals, especially coconut water, are characterized and compared for their similarities and differences to address (1) whether plant exosomes are comparable to exosomes derived from adult stem cell cultures by exerting similar biological activities; (2) whether plant exosomes have any differential advantages or disadvantages as compared to exosomes derived from adult stem cell cultures; and (3) whether plant exosomes can exert cross-species biological activities in humans. By reviewing these questions, the findings in this review could potentially enlighten whether exosomes extracted from plant botanicals, with coconut water in particular, can be an alternative exosomal source to possibly substitute mammalian exosomes.

Part 3: Exosomes Extracted from Plant Botanicals

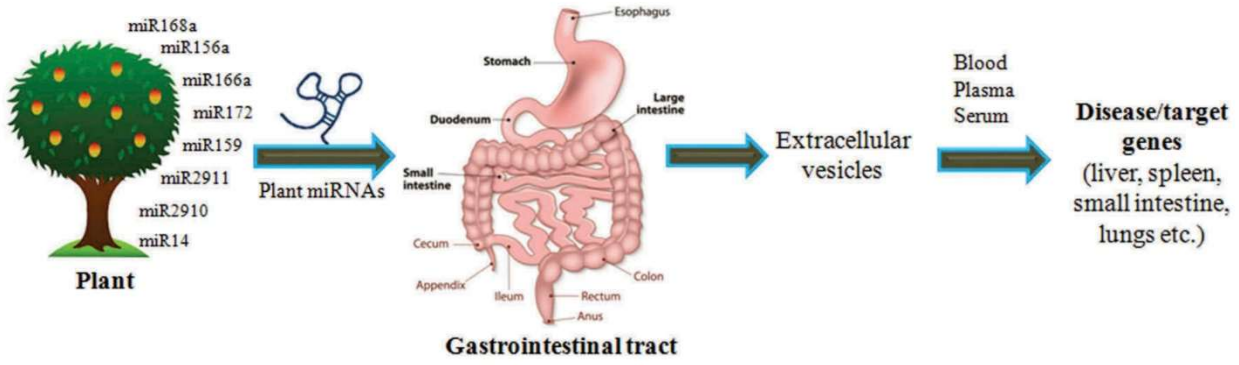
Compared to mammalian exosomes, plant exosomes are characterized for their similarities in exosomal contents [1], morphology and structure [20]. Additionally, plant exosomal cargo can be transferred to human recipient cell via fusion to mediate phenotypic alterations, which makes exosomes dynamic mediators of interspecies intercellular communication, and can regulate endogenous gene expression on recipient human cells [20, 28].

Exosomal microRNAs (miRNAs), found extensively in plants, animals, and humans [20, 28], are short non-coding RNAs that globally regulate gene expression through interaction in a sequence specific manner with their corresponding target mRNAs at both transcriptional and post-transcriptional level, leading to pre- and post-transcriptional gene silencing [29]. Interestingly, approximately 60% of protein coding genes are modulated by miRNAs, which mediate primary and secondary metabolic pathways through cascade of genetic crosstalk by endogenous and/or exogenous regulation [28]. Although the roles of human's miRNAs have been demonstrated to be beneficial in various biological processes such as adipogenesis [30], the highly critical role of plant miRNAs in cross-kingdom gene regulations in humans have received very limited attention. Recently, the miRNAs enriched in edible plant-derived exosome-like nanoparticles (EPDELNs; morphologically similar to exosomes) are found to mediate interspecies intercellular communication such that the molecular profiling has demonstrated exosomal miRNAs can directly inhibit human genes encoding inflammatory factors of interleukin-1, -2, -5, and -6, revealing plant exosomes through enriched miRNAs that can possibly function against cross-species

inflammatory diseases [20]. Additionally, *in silico* studies in plants *Camptotheca acuminata* and *Curcuma longa* have identified miRNAs that target cancer-associated genes in humans, supporting cross-kingdom gene regulation [31, 32].

Importantly, plant miRNAs are characterized for their stability even under the adverse environment in the human gastrointestinal (GI) tract (e.g., RNases, phagocytosis, low pH) due to structural stability of 2'-O-methylation at the 3' end of plant miRNAs [28]. Plant exosomal miRNAs are characterized for their presence in mammalian cells [28] and their biological activities to (1) target and regulate mammalian genes [20]; and (2) specifically bind target mammalian mRNAs and regulate biological processes [33]. This is demonstrated by plant exosomal miRNAs that are not only found to be present human specimens but also are seized by human GI tract, regulating cross-kingdom gene expression in humans and cellular processes through dietary intake [7]. When exogenous plant miRNAs are taken up orally as food intake from raw or cooked form [33], they can pass through GI tract where the plant miRNAs are transferred and packaged into exosomes, which facilitate exosomal miRNAs stability without being degraded, and the exosomal miRNAs are then transferred to human target tissues (e.g., liver, lung) via bloodstream, modulating their target genes expression in humans. (Figure 3) [28]. In other words, the plant exogenous miRNA and host mRNA interactions can alter genetic regulation of host cells where the orally acquired plant miRNAs bind to their complementary targets and modulate the transcriptional/post-transcriptional processes [28].

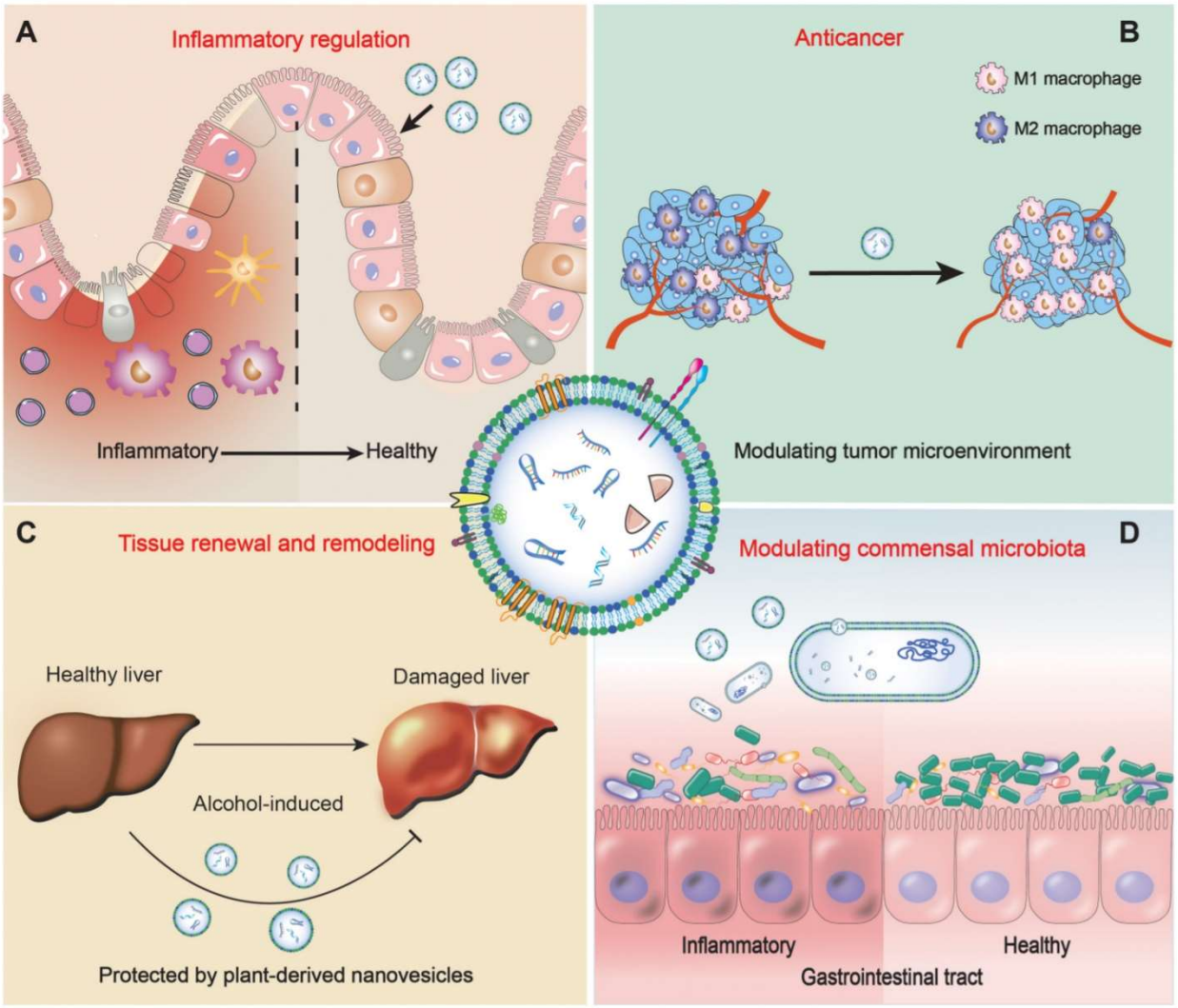
Figure 3: Cross-species regulation of plant exosomal miRNAs on human target genes [28]



Additional characterizations of plant exosomes are inflammation regulation (Figure 4A), anticancer involvement by modulating the tumor microenvironment (Figure 4B), tissue renewal and remodeling to repair alcohol-induced damaged liver (Figure 4C) and commensal microbiota modulation in GI tract (Figure 4D) in human *in vitro* and *in vivo* system [34]. For instance, exosomes extracted from citrus limon can inhibit the proliferation of various solid and hematological cancer cells *in vitro* and suppress chronic myeloid leukemia xenograft tumor growth *in vivo* [34]. Taken together, plant exosomes through exosomal miRNAs can mediate plant-to-

human intercellular communication and cross-species regulation in human target tissues and gene expression.

Figure 4: Biological processes of plant exosomes in regulating (A) inflammation, (B) anticancer, (C) tissue renewal and remodeling, (D) commensal microbiota in human cells *in vitro* and *in vivo* [34]

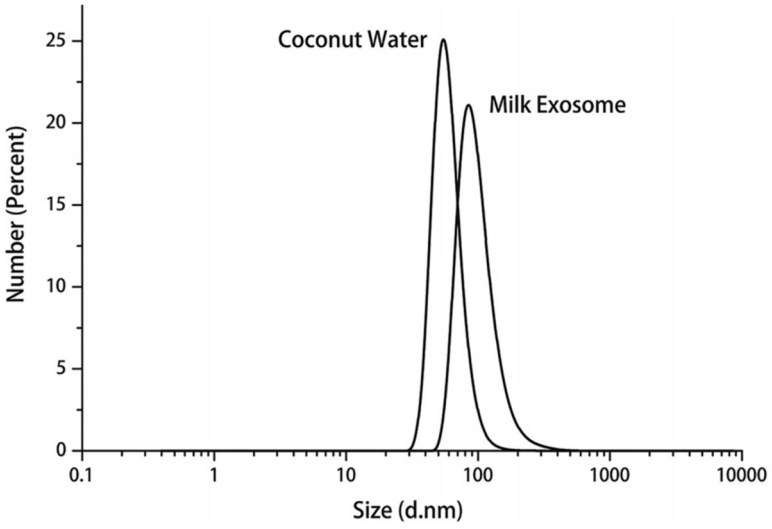


Part 4: Exosomes Extracted from Coconut Water

To compare coconut water and mammalian exosomes regarding exosomal size, exosomes extracted from coconut water are characterized with smaller diameters than those of mammalian exosomes by dynamic light scattering; the average exosomal size in coconut water and milk was 59.72 nm and 100.40 nm, respectively (Figure 5) [21]. In comparison, exosomes derived from adult stem cell cultures found in AF ranged from 50 up to 1000 nm in size, much larger than

coconut water exosomes [35]. Very importantly, not all plant exosomes are characterized of smaller exosomal size than mammalian exosomes. For example, exosomes extracted from ginger are approximately 233 nm [34], even larger than many mammalian exosomes such as milk. Since the smaller exosomal size of coconut water can facilitate better exosomal mobility and more efficient uptake of exosomal contents (e.g., miRNAs) by the recipient cells in humans, such differential advantage of coconut water exosomes is more ideal than exosomes derived from mammalian exosomes and many other plant exosomes, particularly with exosomal size larger than approximately 100 nm, to be used in cross-species coconut-to-humans intercellular communications and regulations on human target gene expression. Compared to exosomes derived from adult stem cell cultures, the smaller diameter characterized by the exosomes extracted from coconut water have key implications: (1) the isolated exosomes from coconut water may have better exosomal mobility and can function as more efficient delivery vehicle upon carrying the exosomal contents (e.g., miRNAs) to the human recipient cells; and (2) higher efficiency in cellular uptake by human recipient cells, which can exert more efficient target biological effects through facilitating cross-species absorption and entry into the circulatory system in humans [21].

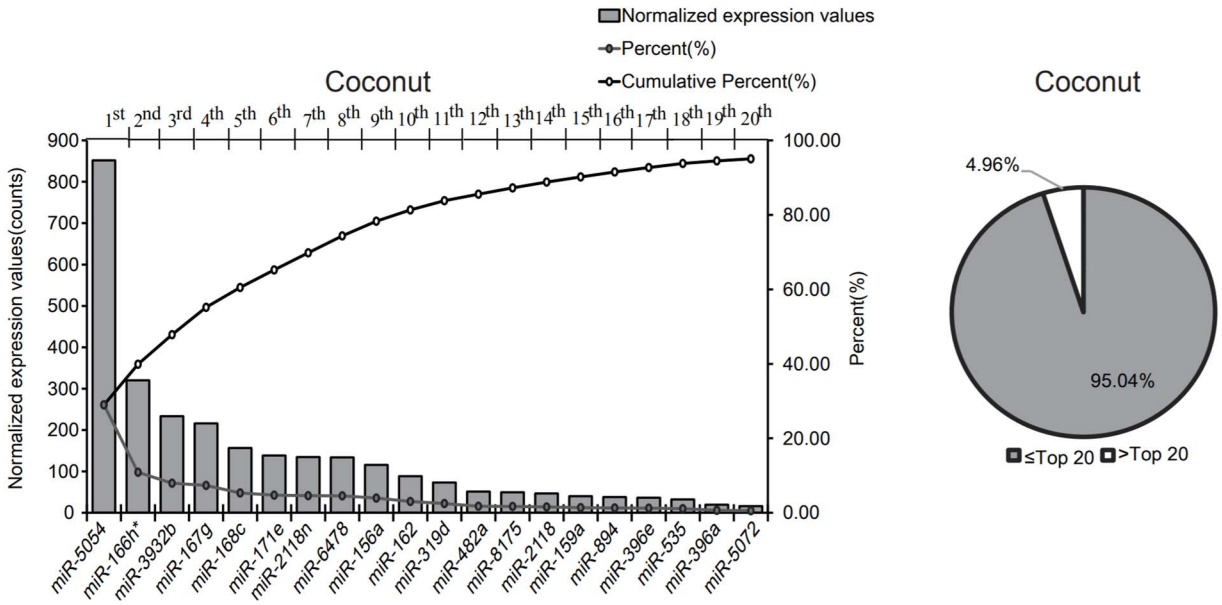
Figure 5: Comparison of exosomal size in coconut water and mammalian exosomes [21]



Although the exosomal miRNA expression levels vary widely cross species, the top 20 exosomal miRNAs in coconut water that are involved in coconut growth and development have contributed greater than 92% of the total miRNA expression, with MIR-167g and MIR-2118n have identified to be the most highly expressed miRNAs unique to exosomes isolated from coconut water over other plant species (Figure 6) [20]. The normalized expression values and proportions of exosomal miRNAs in coconut water have revealed that the exosomal miRNAs of coconut water can possibly regulate human target genes through base complementary pairing between the exosomal miRNA of coconut water and human target genes, suggesting exosomal miRNAs of coconut water can

specifically bind target human mRNAs and influence biological processes in human cells [20]. For example, long-term oral intake of coconut water that contain exosomal miRNA, MIR-159, are found to inhibit human breast tumor growth using in vitro and in vivo models by targeting transcription factor 7 (TCF7) that encodes a Wnt signaling transcription factor, leading to decrease in MYC protein levels through exosomal miRNA-mediated modulation of tumor microenvironment [33].

Figure 6: Expression profiles of exosomal miRNAs in coconut water [20]

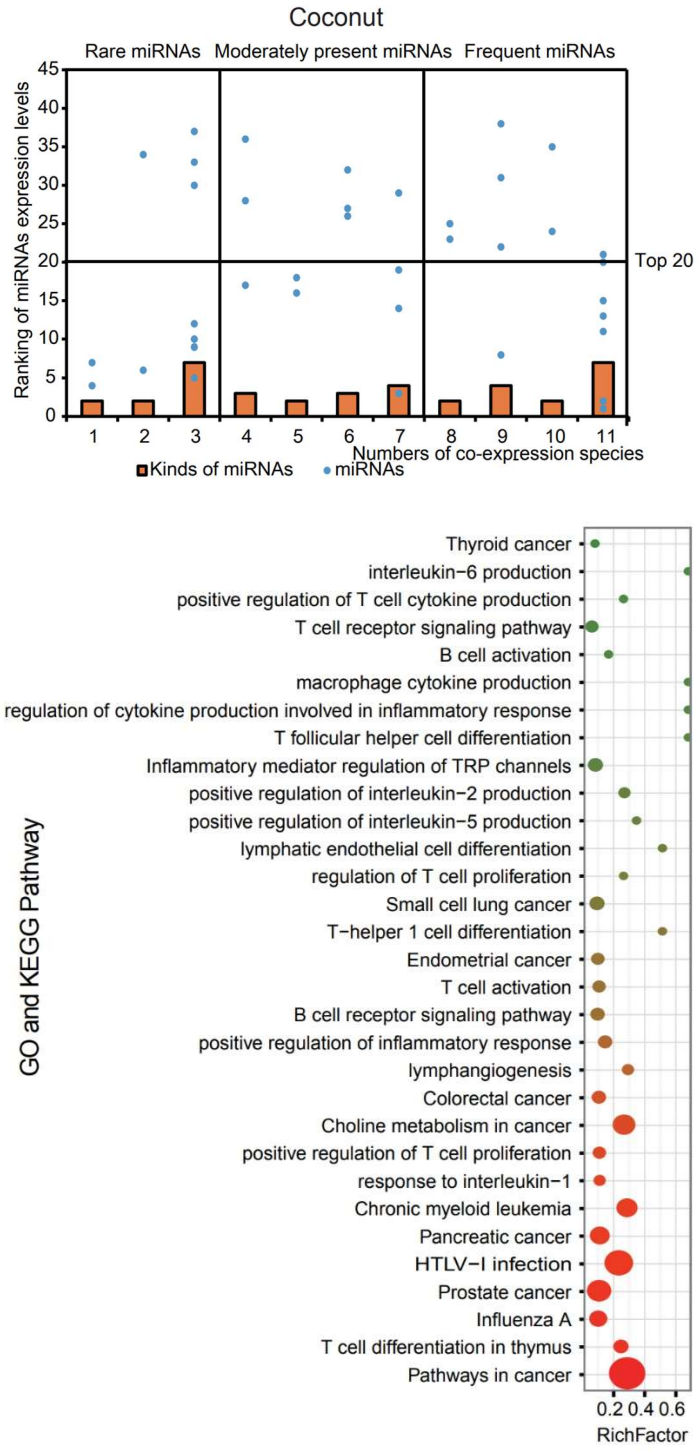


Species	Scientific name	miRNA	Sequence	Normalized expression values	Rank
Coconut	*Cocos nucifera*	MIR-167g	TGAAGCTGCCAGCATGATCTGA	216.16	4
		MIR-2118n	TTCCCGATGCCTCCCATTCCTA	134.62	7

Plant exosomal miRNAs are found to specifically bind target mammalian mRNAs, which can alter biological processes [33]. Consistently, exosomal miRNAs from coconut water is further characterized by bioinformatics analysis using TargetScan to predict relationships between exosomal miRNAs of coconut water and their corresponding human target genes based on the principle of base complementary pairing between the plant miRNA and their target human genes (Figure 7) [20]. Such target gene prediction has supported that miRNAs can target and regulate human target genes, as demonstrated by the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses (http://www.genome.jp/kegg), indicating that the target genes of miRNAs are associated with immune cells and are significantly enriched in cancer-related signaling pathways (Figure 7) [20]. Additionally, frequent miRNAs are

found to be represented by fewer miRNA species than rare miRNAs but had a significantly higher cumulative expression level, and such highly expressed miRNAs in coconut water can regulate the expression of human inflammatory cytokines and cancer-associated genes *in vitro* [20].

Figure 7: Expression distribution of exosomal miRNAs in coconut water [20]



Interestingly, the average increase in miRNAs has found to be approximately 20 times higher in immature coconut water than in mature coconut water, with the highest degree of change in miR528-5p for 112 folds higher, demonstrating biological process of possibly regenerative potentials in immature coconut [21]. The immature (young) coconut resembles the young mouse from the heterochronic pair in the old-young mice parabiosis experiment in which the young mouse contains the young serum that promote cell proliferation [36] such that the young coconut water is characterized with the much higher exosomal miRNA-mediated biological processes that promote its proliferation. From studying target gene prediction in coconut water, possible PI3K/AKT and MAPK/ERK signaling pathways might be associated with cell growth, metabolism, and disease, further demonstrating that exsosomal miRNAs from coconut water can alter human gene expression.

Overall, the aforementioned biological effects of exosomes extracted from plant botanicals, including specific illustrations of exosomal activities in coconut water, are recognized to be beneficial and comparable/similar to exosomes derived from adult stem cell cultures as demonstrated by (1) the exosome-mediated intercellular communicate by plant exosomes could also exert potent cellular proliferation, differentiation, and immunomodulatory capacity, as demonstrated by *in vitro* and *in vivo* systems, similar to exosomes derived from adult stem cell cultures; and (2) exosomes extracted from plant botanicals especially coconut water can exert cross-species plant-to-human intercellular communication, ultimately effecting similar biological activities to alter target human gene expression *in vitro*. Besides their similarities, plant exosomes have demonstrated several differential advantages, including: (1) coconut water, along with other plant exosomes, are characterized of fully natural constituents from both the plant exosomes themselves and the natural constituents that they carry in the cargo, and such natural source of exosomes extracted from plant botanicals may enhance bioavailability and minimize potential allergenic effects and immunogenicity; (2) plant exosomes, with coconut water in particular due to smaller exosomal size and exosomal isolation convenience, are ideal source of exosomes through targeting human cell proliferation, regeneration, differentiation, and modulating tumor microenvironment by cross-species intercellular communication.

Conclusion

This review advances the field of regenerative medicine through reviewing alternative exosomal sources extracted from plant botanicals especially coconut water by characterizing and comparing exosomes derived from adult stem cell cultures. Overall, this review has demonstrated: (1) plant exosomes, especially coconut water, are comparable to exosomes derived from adult stem cell cultures by characterizing with similar exosomal structures and exosomal compositions as well as exerting similar biological activities against human cell proliferation, differentiation and regeneration in human systems, at least revealed by the *in vitro* systems; (2) plant exosomes can exert cross-species plant-to-human biological activities through plant exosome-mediated intercellular communications in humans; (3) plant exosomes have differential advantages due to

their natural, biodegradable, renewable and sustainable resources as compared to exosomes derived from adult stem cell cultures; and (4) exosomes isolated from coconut water are even better exosomal sources due to their exosomal isolation convenience and smaller exosomal size that are more easily absorbed by human circulatory system. The findings in this review support that exosomes extracted from plant botanicals, especially coconut water, can be used to not only potentially substitute exosomes derived from adult stem cell cultures but also introduce a possibly better alternative due to their differential advantages of plant exosomes over exosomes derived from adult stem cell cultures, especially the latter are challenged by the scalability in large-scale applications due to limited resources and supplies [17]. Future investigations to explore the efficacy and safety profile of plant exosomes in humans are yet to be further tested through clinical trials.

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Author Profile

Tong Shen, M.D., is a graduate of the Blue Marble University School of Medicine and St. John's University College of Pharmacy and Health Sciences. She is an oncology medical lead and clinical development professional across clinical trial Phase I to IV for multi-indication oncology programs leading to successful multinational registrations and launching readiness at pharmaceutical/biotech industries. She is also a key contributor and author of 15 publications in oncology journals and one patent in biometric device. Her career contributions span nearly 15 years.

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